REMARKS/ARGUMENTS

Claims 13-16, 20-24, 27-32, 36-44, and 48-52 are pending in the present application. The pending claims have been amended to recite specifically that the claimed amino acid sequence is in the polymerase domain of the DNA polymerase. Support for this language is found, for example, at page 12, lines 34-37. There it is explained that the claimed motif "identifies a particular functional region within the polymerase domain of the enzyme, and identifies an amino acid within the motif that is critical to the function." In addition, Table 1 on page 12 shows the location of the claimed motif in a number of polymerase enzymes. No new matter is added with this amendment. The claims have been amended only to expedite prosecution. Applicants specifically reserve the right to pursue the un-amended claims in one or more subsequent applications.

The claims stand rejection under 35 U.S.C. § 102(b) as allegedly being anticipated by Bergquist *et al.* (WO 95/14770).

According to the Office Action, this reference discloses a *Thermus filiformis* DNA polymerase allegedly having reverse transcriptase activity in the presence of magnesium and comprising the motif disclosed in SEQ ID NO: 1. As noted previously, the pending claims are directed to ***mutated*** DNA polymerases, which in their ***native*** form comprise the recited sequences. Indeed, the Examiner acknowledges the scope of the pending claims by stating that the previous rejections are withdrawn in view of applicant's arguments. As stated in the Office Action at page 2, paragraph 3(a), the pending claims are not directed to polymerases that comprise the recited sequences, "but rather require an enzyme ***which has been altered from this sequence***." (emphasis added).

The *T. filiformis* DNA polymerase disclosed by Bergquist *et al.* is a ***wild-type*** enzyme that comprises the recited sequences. The subsequence referred to by the Examiner **(LSDRIHLLHPE)** is present in the wild-type enzyme, as clearly shown in the Figure 1 of the Bergquist *et al.* publication. Since, as acknowledged by the Examiner, the claims are directed to enzymes in which residues within the critical motif are ***mutated*** to the recited sequences, the

claims do not encompass this wild-type enzyme. Thus, on this basis alone the rejection is improper and should be withdrawn.

Moreover, the subsequence identified in the Office Action is not the motif disclosed and claimed here. As explained at page 12, the motif claimed here is present in the ***polymerase domain*** of the wild-type polymerase (*see* page 12, lines 34-37). Page 13, lines 18-28 of the specification shows that the location of the motif can be easily determined by comparison of the sequence to a DNA polymerase known to have the motif. Indeed, the location of the claimed motif in *T. filiformis* is identified in Table 1, as being at position 679 and having the sequence LSQELSIPYEE.[1] The motif can be seen in the Bergquist *et al.* publication in Figure 1-3, on the lines beginning with residues 1891 and 2041, respectively. Thus, the claimed motif present in the wild-type enzyme does not comprise a residue other than E, A, G, or P at position 4, as required by the pending claims. Since Bergquist *et al.* fails to disclose a DNA polymerase (either wild-type or mutated) with the motif as claimed here, it cannot anticipate the pending claims.

To further clarify this distinction and to expedite prosecution, the pending claims have been amended as noted above. The claims now specifically recite that the claimed motif is present in the polymerase domain of the DNA polymerase. In light of the above, applicants respectfully submit that these claims are clearly distinguished from the cited prior art and the rejection should be withdrawn.

Finally, with regard to the reliability of the experimental results shown in Berquist *et al.*, the Examiner asserts that Figure 7 (allegedly showing reverse transcription of a serial dilution of α-lactalbumin mRNA) and Figure 8 (allegedly showing reverse transcription of topoisomerase IIa mRNA or α-lactalbumin mRNA from total RNA) provide actual results showing that reverse transcription did occur. On page 12, Bergquist *et al.* disclose two forward primers (T7 and P3) and two reverse primers (P1 and P2) allegedly used in these experiments. As explained below, these four primers cannot be used to reverse transcribe or amplify either of the two molecules allegedly exemplified there.

[1] The critical amino acid residue at position 4 of the motif is highlighted.

As explained previously, one of the forward primers, T7, clearly could not be used in the experiments, because it is derived from the T7 promoter sequence, which is absent from mRNA transcripts. The Examiner correctly points out , however, that the other forward primer, P3, could have been used, instead. The P3 sequence (5' TAT TCC CAA ACT GGA TGA TGC TAA T 3') was searched against NCBI nucleotide database using BlastN. As shown in the highlighted portion of the attached sequence comparison, P3 is derived from the human topoisomerase II**b** gene (Exhibit 1, highlighted portion). Surprisingly, however, Bergquist *et al.* assert that mRNA from the human topoisomerase II**a** gene was the template in the experiments. The reverse primer P2 sequence ('5 ATT TTC CAT GAT CTG CTT ATG AG 3') was searched against NCBI nucleotide database using BlastN. These results show that P2 appears to hybridize to the topoisomerase II**a** gene (Exhibit 2, highlighted portion). This combination of primers, however, clearly could not reverse transcribe mRNA from either the topoisomeras IIa or IIb gene since they are derived from two different genes. Since Bergquist *et al.* do not provide any sequence information for the amplification products there is no way to exclude the possibility that the detected bands are the result of amplification of contaminating DNA in the RNA samples.

With regard to reverse transcription of α-lactalbumin mRNA shown in Figure 7, only the reverse primer P1 is derived from α-lactalbumin (*see*, Exhibit 3, which shows the results of the sequence 5' CGG ATC CCA AAT CAG GCT TTT ATT CGG 3' searched against NCBI nucleotide database using BlastN). No other primer referred to on page 12 would hybridize to this transcript.

Assuming that the primer pair used in these experiments was T7 and P1,[2] the results actually provide evidence that, in fact, the RNA samples were contaminated with plasmid DNA. The experiments are reported to have been carried out using an RNA transcript of α-lactalbumin (*see* page 9, brief description of Figure 7). As explained on page 11, the α-

[2] Bergquist *et al.* never identify which primers are used in which experiment.

lactalbumin RNA was derived from *in vitro* transcription of a cloned α-lactalbumin gene, presumably linked to a T7 promoter. If plasmid DNA contaminated the RNA in the experiments, the plasmid DNA would have been a template for an amplification reaction using these two primers. Accordingly, the strong bands in Figure 7 could easily be derived from contaminating plasmid DNA.

The above discussion clearly shows that there is no combination of primers disclosed by Bergquist *et al.* that could be used to reverse transcribe ***any*** of the mRNA molecules described there. The results in Figures 7 and 8 cannot be believed to demonstrate efficient Mg^{2+}-activated reverse transcriptase activity by a *T. filiformis* DNA polymerase, because that is simply impossible, given the experimental procedures disclosed there. Indeed, to the extent they show some kind of amplification reaction, it is likely that the detected bands are products of plasmid DNA contamination of the RNA samples.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,

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